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PDR5, a Novel Yeast Multidrug Resistance Conferring Transporter Controlled by the Transcription Regulator PDR1*

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The complete sequence of the pleiotropic drug resistance gene *PDR5* from *Saccharomyces cerevisiae* is reported and analyzed. *PDR5* encodes a 160-kDa protein with a predicted duplicated six-membrane-span domain and a repeated putative ATP-binding domain. *PDR5* shares this structural feature with the mammalian multidrug resistance pumps as well as the functional capacity of conferring resistance to various inhibitors upon amplification (Leppert, G., McDevitt, R., Falco, S. C., Van Dyk, T. K., Ficke, M. B., and Golin, J. (1990) *Genetics* 125, 13-20). The yeast *PDR5* is thus a new member of the ABC (ATP-binding cassette) protein superfamily.

Mutations in another yeast pleiotropic drug resistance gene, *PDR1*, encoding a putative transcription regulator (Balzi, E., Chen, W., Ułaszewski, S., Cappelaux, E., and Goffeau, A. (1987) *J. Biol. Chem.* 262, 16871-16879), increase markedly the mRNA levels of the *PDR5* and *STE6* genes. The multidrug resistance mutations *pdr1-3* and *pdr1-6* also lead to considerable overexpression of the *PDR5* plasma membrane protein.

and antiseptics have been clustered in a drug-resistance conferring subfamily, which also comprises the yeast aminotriazole-resistance determinant *ATR1* or *SNQ1* (Kanazawa et al., 1988; Gömpel-Klein and Brendel, 1990) and three newly discovered homologous proteins encoded by *Saccharomyces cerevisiae* chromosome III genes (Goffeau et al., 1993).

The ABC superfamily (review by Higgins (1992)) comprises over 50 proteins from bacteria to man sharing a basic structure of two six-membrane span hydrophobic domains and two large hydrophilic domains, each containing a conserved ATP-binding cassette. This architecture has been associated with both active transport and passive ion channel activities (review by Ferro-Luzzi Ames and Lecar (1992)).

One of the most studied ABC transporters is the mammalian P-glycoprotein, responsible for pumping drugs out of the cell and for resistance of cancer cells toward various antineoplastic drugs (reviews by Juranka et al. (1989), Endicott and Ling (1989), and Shinkel and Borst (1991)). Protozoan homologues of the P-glycoprotein seem to be responsible for the resistance acquired by *Plasmodium falciparum* toward the antimalaria drug chloroquine (Foote et al., 1989) and of *Leishmania tarentolae* toward methotrexate and heavy metals (Ouellette et al., 1990). Similar ABC-like ATPases from bacteria are involved in the export of toxic ions (Silver et al., 1989), antibiotics (del Carmen Garrido et al., 1988), and anti-cancer drugs (Guilfoile and Hutchinson, 1991; Miyauchi et al., 1992). The ABC protein's superfamily includes also the yeast *STE6* pump for secretion of the mating pheromone a (McGrath and Varshavsky, 1989; Kuchler et al., 1989) and the human cystic fibrosis chloride channel CFTR (Riordan et al., 1989).

A common mechanism underlying multidrug resistance is overexpression of the MDR (multidrug resistance) P-glycoproteins which, by driving the drugs out of the cell, reduces their cytoplasmic concentration and, hence, toxicity (Shinkel and Borst, 1991). Some MDR genes become overexpressed in multidrug-resistant cancer cells, probably reflecting general regulatory disorders occurring during tumorigenesis (Chin et al., 1992). However, little is known about the molecular mechanisms by which the MDR genes are regulated.

A phenotype corresponding to mammalian multidrug resistance has been reported for almost three decades in yeast cells, which often provide reliable and convenient model for unraveling eukaryotic pathways. At the basis of yeast multidrug resistance, one or most probably several putative networks comprising a total of no fewer than 20 genes has been outlined (Balzi and Goffeau, 1991; Meyers et al., 1992; Dexter et al., 1993). Among them, some transcription regulator factors, such as *PDR1* (Balzi et al., 1987), *PDR3* (Delaveau et al., 1992), and *YAP1/SNQ3/PAR1/(PDR4)* (Hussain and Lenard, 1991; Moye-Rowley et al., 1989; Hertle et al., 1991; Schnell et al., 1992) were recently identified and sequenced.

Resistance to multiple cytotoxic compounds is an acquired property in many species from bacteria to man. The major molecular determinants mediating multidrug resistance are transport proteins driving the traffic of drugs and physiological substrates across the cell membrane. The drug-resistance conferring proteins identified so far can be classified within two superfamilies: the major facilitators superfamily and the ATP-binding cassette superfamily (ABC).¹

The major facilitators superfamily (Marger and Saier, 1993) is characterized by a structural motif of "2 × 6" transmembrane α -helices with a dispensable central cytoplasmic domain. Among the more than 50 uni-, sym-, and antiporters of sugars, organic acids, or drugs belonging to this superfamily, several bacterial proteins catalyzing the efflux of various drugs

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¹ The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L19922.

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The abbreviations used are: ABC, ATP-binding cassette; MDR, multidrug resistance; PDR, pleiotropic drug resistance; kb, kilobase(s); bp, base pair(s); STE, sterility; CFTR, cystic fibrosis transmembrane conductance regulator.

The target genes of these regulators have not been identified yet at the molecular level. However, recent genetic evidence points to a relation between the PDR1 regulator and the PDR5 gene (Meyers *et al.*, 1992).

In this paper, we elucidate the nature of the yeast gene *PDR5*, a locus conferring multidrug resistance upon amplification and extreme hypersensitivity to a variety of inhibitors upon inactivation (Leppert *et al.*, 1990). The analysis of its nucleotide sequence reveals that it encodes a putative membrane pump belonging to the ABC superfamily that is homologous to the mammalian P-glycoprotein. We also report that mutations of the yeast transcription regulator factor, PDR1, provide considerable overexpression not only of the *PDR5* transcript but also of the PDR5 protein located in the plasma membrane.

EXPERIMENTAL PROCEDURES

Yeast Strains—The *PDR5* gene was sequenced from *S. cerevisiae* S288C (*MATa*, *SUC2+*, *gal2*, *mal0*). The following *S. cerevisiae* strains were also used: DRI9-T8 (*MATa*, *pdr1-3*, *his1*), IL125-2B (*MATa*, *his1*; parental of DRI9-T8), D1-1 (*MATa*, *pdr1Δ::URA3*, *ura3*, *his1*), US53-31D (*MATa*, *pdr1-1*, *ura3*, *his1*; host for disruption of *PDR1* and parental of D1-1); D1 (*MATa*, *pdr1Δ::URA3*, *ura3*), 2295-C (*MATa*, *ura3*; host for disruption of *PDR1* and parental of D1) (Balzi *et al.*, 1987 and references therein); US89-13B² (*MATa*, *pdr1-6*, *ura3*, *ade1*); BOR2-XI (*MATa*, *pdr1-6*, *ade1*, *his1*), D286-2A (*MATa*, *ade1*, *his1*; parental of BOR2-XI) (Nass and Poralla, 1976).

Subcloning and Sequencing of the *PDR5* Gene—A 6.6-kb *Sau3AI-PvuII* genomic DNA fragment from *S. cerevisiae* S288C, comprising the *PDR5* gene and representing the 5'-half of the *Sau3AI* insert of plasmid pDR3.3 (Leppert *et al.*, 1990) was sequenced. After sonication of pDR3.3, the extremities of fragments ranging from 0.5 to 1.5 kb were filled in by T4 DNA polymerase and Klenow fragment and then ligated to the *Sma1* site of the pSK⁺ or pSEQ1³ sequencing vectors. Subclones of interest were selected by *in situ* colony hybridization to four nick-translated restriction fragments from the 6.6-kb *Sau3AI-PvuII* region. These *PvuII* fragments of 4.7 and 0.5 kb and *EcoRI* fragments of 0.8 and 0.5 kb, used as probes for hybridization, were previously cloned in the *Sma1* and *EcoRI* sites of pSEQ1, respectively. Bacterial transformations were performed in the *Escherichia coli* strain JM109. Double strand DNA from positive clones was sequenced according to the dideoxy chain termination method (Sanger *et al.*, 1977) using T7 DNA polymerase (Pharmacia LKB Biotechnology Inc.) and M13 forward and reverse primers. Where necessary, synthetic oligonucleotide primers were used to fill in data gaps. The entire sequence was determined on both strands. The sequences were assembled and analyzed with the PC/GENE software (Intelligenetics, Inc.). Searches for homology, using the FASTA algorithm (Pearson and Lipman, 1988), were carried out in the PIR1 (release 30) and MIPSX (release 30.2) protein data bases at the Martinsried Institute for Protein Sequences (Munich, Germany).

RNA Analysis—The 5' termini of the *PDR5* transcript have been mapped by the S1 nuclease method described by Sambrook *et al.* (1989). The radiolabeled double strand DNA of the *HindIII-BgIII* fragment, covering the positions -360 to +13 with respect to the *PDR5* start codon, were used as a probe for hybridization to total mRNA from the strain DRI9-T8. Northern blot analyses of transcript levels were performed as previously described (Balzi *et al.*, 1987) by hybridization to probes specific for the *PDR5* and *STE6* genes, derived, respectively, from the plasmids pDR3.3 (Leppert *et al.*, 1990) and pUC-STE6 (McGrath and Varshavsky, 1989).

Protein Preparations and Analysis—Plasma membrane-enriched protein fractions were prepared as described by Goffeau and Dufour (1988) except that no vanadate was used in the grinding medium. Cells from a 1 liter (2% yeast extract, 5.8% glucose) culture in exponential growth phase were mechanically disrupted and centrifuged 3 times at 1,000 \times g for 5 min to eliminate cell walls and unbroken cells and then at 15,000 \times g for 40 min to obtain a crude membrane pellet. The mitochondrial and ribosomal components were then separated from the plasma membranes by selective acid precipi-

tation at pH 5.2. The different protein fractions obtained were analyzed by polyacrylamide gel electrophoresis (Laemmli, 1970) stained by Coomassie Blue. For NH₂-terminal protein sequencing, about 450 μ g of protein from plasma membrane-enriched fractions of the *pdr1-6* strain US89-13B were run on a 6% acrylamide minigel, electroblotted on a polyvinylidene difluoride-type membrane (Immobilon P., Millipore) for 2 h at 50 V, and stained with Amido Black. The overexpressed fuzzy band was cut from the membrane and submitted to NH₂-terminal microsequencing in a pulsed-liquid phase sequenator (model 477A, Applied Biosystems, Inc.) equipped with an on-line phenylthiohydantoin amino acid derivative analyzer (model 120A).

RESULTS

***PDR5* Encodes an ABC Protein**—The *PDR5* gene had been previously cloned as a multicopy plasmid-borne DNA fragment capable of conferring multidrug resistance (Leppert *et al.*, 1990). Transposon inactivation analysis of the 14.7-kb DNA insert containing *PDR5* had pinpointed a 6.6-kb subregion as being responsible for the multicopy-mediated multidrug resistance (Leppert *et al.*, 1990). The nucleotide sequence of this 6552-bp fragment revealed an open reading frame of 4533 bp and 5'- and 3'-flanking regions of 1611 and 408 bp, respectively (Fig. 1). In the *PDR5* 5'-flanking region, three main sites of transcription initiation were identified by S1 mapping at about 171, 174, and 175 bp upstream from the translation start (data not shown). Other typical features observed in the 5'-flanking region of *PDR5* are: an adenine at position -3, a TATAATA box (Chen and Struhl, 1988) about 60 bp upstream (position -234) from the estimated transcription starts, and two sequences corresponding to the binding consensus elements for the heat shock factor (Pelham, 1985) located 679 and 663 bp upstream from the start codon, respectively. In the 3'-flanking region of *PDR5*, a sequence matching the polyadenylation signal AATAAA (Proudfoot and Brownlee, 1976) was observed 54 bp downstream from the stop codon. The transcription terminator signals described by Zaret and Sherman (1982) (+4773: TAGN₁, TAGTN₂, TTT) or by Henikoff and Cohen (1984) (+4769: TTTTATA) were detected about 240 bp downstream from the translation stop.

The *PDR5* gene encodes a polypeptide of 1511 amino acids (Fig. 1) if the most upstream in-frame ATG is considered as start codon. This position is confirmed by the NH₂-terminal amino acid sequence of the PDR5 protein, determined as described below. The PDR5 polypeptide has a calculated molecular mass of 170,437 Da and an expected isoelectric point of 7.7. According to the method of Klein *et al.* (1985), PDR5 is predicted to contain nine "integral" transmembrane spans (threshold value for P:I odds < 1) and three "possibly integral" spans (threshold value for P:I odds < 1.5 \times 10), gathered in two groups of six contiguous membrane spans. Each hydrophobic domain follows a hydrophilic region including a predicted ATP-binding cassette. PDR5 appears thus to have a duplicated structure, consisting of two halves composed each of one hydrophilic and one hydrophobic domain. A topographical model for the orientation of PDR5 in the membrane is proposed in Fig. 2.

The two ATP-binding cassettes of PDR5 are similar to those conserved in the ABC superfamily of transport proteins (Hyde *et al.*, 1990; Mimura *et al.*, 1991; Ferro-Luzzi Ames and Lecar, 1992; Thomas and Pedersen, 1993), consisting of a domain of about 200 amino acids and comprising the ATP-binding motifs A and B of Walker (1982) and, just preceding the Walker B motif, a conserved sequence termed the ABC signature. The COOH-terminal ABC cassette of PDR5 contains two well conserved A and B Walker's motifs, although no typical ABC signature is observed. The NH₂-terminal ABC

² Kind gift of S. Ulaszewski, University of Wroclaw, Poland.

³ Derivative of pGEM-3Zf, kindly donated by J. C. Jauniaux, Université Libre de Bruxelles, Belgium.

FIG. 1. Nucleotide and deduced amino acid sequence of *PDR5*. The nucleotide and amino acid sequences of a 6552-bp *Sau3A1-PvuII* fragment containing the *PDR5* gene are numbered to the *left* and *right* side, respectively. The estimated transcription initiation sites are indicated by arrows. Consensus sequences for binding of the heat shock factor (HSE) as well as polyadenylation consensi are underlined. A TATA sequence is *double underlined*. In the 3'-flanking region, the transcription termination signals of Henikoff and Cohen (1984) and Zaret and Sherman (1982) are marked respectively by · and ¸. In the amino acid sequence, membrane-spanning segments are *underlined*, the Walker's A and B and the ABC signature motifs are *double underlined*; the degenerated A motif is marked with *dashes*. Potential *N*-glycosylation sites (•) and phosphorylation sites by cAMP-dependent protein kinase (Δ) and protein kinase C (\blacklozenge) are also indicated.

FIG. 1—continued

cassette shows a well conserved ABC signature, but the A and B Walker's motifs are degenerated: in the A region, the conserved lysine is replaced by a cysteine; in the B region, the conserved aspartate residue is not preceded by the typical 4 hydrophobic residues. However, an alignment of the complete ABC cassettes of PDR5 shows 74% similarity between these two domains (Fig. 3).

A sequence comparison in protein data banks revealed homologies between PDR5 and several members of the ABC transport superfamily. The three best alignments were obtained with the yeast ADP1 (40.4% identity in 173-amino acid overlap) and *Drosophila* White (28.6% identity in 553-amino acid overlap) and Brown (33.1% identity in 172-amino acid overlap) proteins. The region of homology is centered on the ABC cassettes, in particular the COOH-terminal one. An alignment of the A and B regions of PDR5 with some of the other main ABC transporters is presented in Fig. 4. Recently, the sequence of a new yeast drug-resistance conferring ABC transporter, termed SNQ2, has been reported (Servos *et al.*, 1993), which displays 37% identity and 50% similarity throughout the entire lengths of the two proteins⁴ (Fig. 5). PDR5 and SNQ2 have comparable amino acid chain lengths of 1511 and 1502 residues, respectively. They share a similar domain organization, with the repeated alternation of a hydrophilic and a hydrophobic domain, and a highly similar primary structure in the ABC regions, including the conservation of rare elements such as the cysteine of the NH₂-terminal A motif and the COOH-terminal ABC signature (Fig. 4). PDR5 also displays 10 putative *N*-glycosylation sites, 13 phosphorylation sites for protein kinase C, and 3 putative sites for cAMP-cGMP-dependent protein kinase. These latter are mainly located in the hydrophilic and predicted cytoplasmic regions (Fig. 2).

The PDR5 Gene Shares with the STE6 Gene a Transcriptional Control by the PDR1 Gene Product—In *S. cerevisiae*, multidrug resistance can result from mutations in the *PDR1* gene encoding a transcription regulator-like protein (Balzi *et al.*, 1987) proposed to control the expression of various membrane transporters (Balzi and Goffeau, 1991). *PDR5* is an excellent candidate for a transporter to be regulated by *PDR1*

(Leppert *et al.*, 1990; Meyers *et al.*, 1992). Other putative membrane pumps are: STE6, which secretes the α mating factor and is highly homologous to the mammalian multidrug-resistance conferring P-glycoprotein (McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989); ADP1, an ABC-like transporter (Purnelle *et al.*, 1991); ATR1 or SNQ1, a putative multidrug membrane pump (Kanazawa *et al.*, 1988; Gömpel-Klein and Brendel, 1990), and YGL022, a membrane transport-like protein (Chen *et al.*, 1991) involved in resistance to drugs (Sauer, 1992).

We have analyzed, by Northern blot, the transcript levels of these genes in multidrug-resistant *pdr1* mutants. As shown in Fig. 6 and in a recent publication (Meyers *et al.*, 1992), the 5.2-kb *PDR5* transcript is dramatically overexpressed in multidrug-resistant mutants carrying the alleles *pdr1-6* and *pdr1-3*. The *PDR1* gene has been disrupted by deletion of a 0.6-kb fragment in the 3'-half of the gene (*pdr1Δ::URA3*), and this disruption has been associated with a drug-hypersensitive phenotype (Balzi *et al.*, 1987). When this *pdr1Δ*-disrupted allele is used to replace the *pdr1* mutant alleles, *pdr1-1* (Fig. 6) or *pdr1-3*,⁵ a decrease of the *PDR5* transcript level is observed (Fig. 6). When the *pdr1Δ::URA3* null allele is used for replacement in a wild type (*PDR1*) strain, which has a low basal level of *PDR5* mRNA, no decrease of *PDR5* transcript can be detected by Northern blot analysis.

Fig. 6 shows that the level of *STE6* transcript is also increased in the mutant *pdr1-6*. No *STE6* mRNA is detected either in the *pdr1-3* mutant or in its parent, as expected for strains of mating type α , which normally repress the expression of the *STE6* gene. In contrast to *PDR5*, the level of *STE6* transcript is not affected after disruption of *PDR1*, either in a mutant (*pdr1-1*) or wild type background.

The transcripts of *ADP1* and *ATR1* were not found to be affected in *pdr1* mutants,⁶ while a slight decrease of the *YGL022* transcript was observed in a *pdr1-3* mutant.⁷

Overexpression of the *PDR5* Protein in Plasma Membranes from *pdr1* Mutants—We investigated whether the overexpression of *PDR5* and *STE6* transcripts in *pdr1* mutants was

⁶ B. Jacques and E. Balzi, unpublished observations.

⁶ E. Balzi and B. Jacques, unpublished observations.

⁷ E. Capieaux, personal communication.

⁴ S. More-Rowley, personal communication.

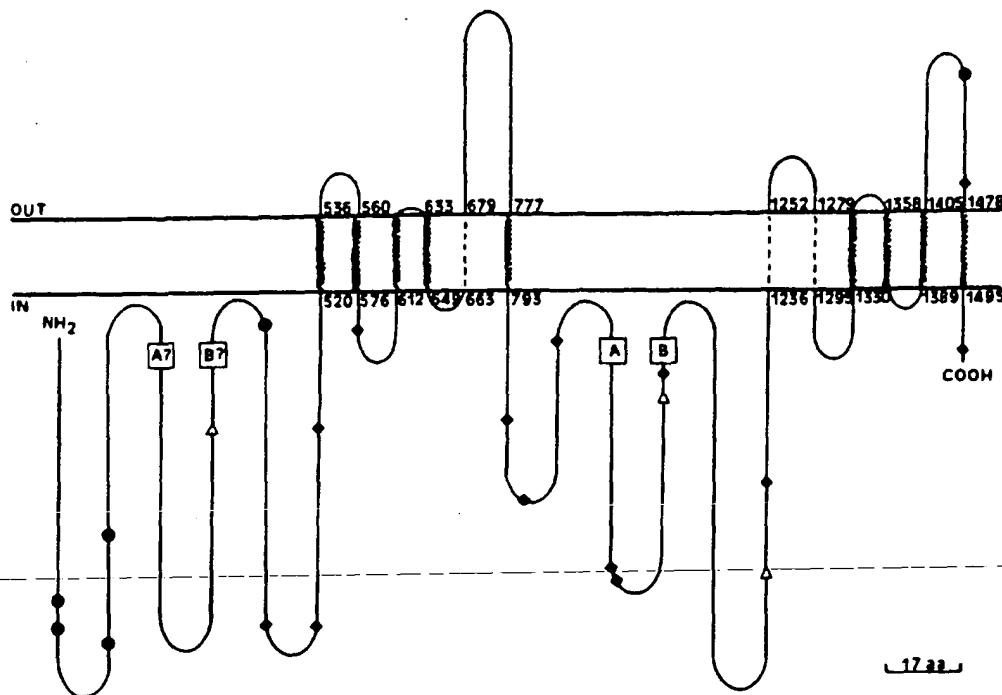


FIG. 2. Predicted topology of the PDR5 protein. The predicted PDR5 protein consists of two halves, each comprising one hydrophilic domain, predicted to be cytoplasmic (IN), followed by one hydrophobic domain, integrated in the plasma membrane through six membrane spans (—, integral transmembrane spans; ---, possibly integral spans with P:I odds $< 1.5 \times 10$ (Klein *et al.*, 1985)). The hydrophilic domains comprise the Walker's A and B ATP-binding motifs (?), degenerated motif). Putative N-glycosylation sites (●) and phosphorylation sites by protein kinase C (◆) and cAMP/cGMP-dependent protein kinase (△) are indicated.

FIG. 3. Alignment of the two ABC cassettes of PDR5. Identical residues (•) and conservative substitutions (.) in the alignment of the NH₂-terminal (N) and COOH-terminal (C) ATP-binding cassettes of PDR5 are indicated. The Walker's A and B motifs, as well as the ABC-transporter signature (S) are boxed. Alignment was made by the program ALIGN (Myers and Miller, 1988).

A	
PDR5 N	163 KFQRSKEITWTPQIILKPMQDGCLNPGEVLLVVLGRPGSGCTTLLKSISSSNTHGFDLGADTKISYSGYS
PDR5 C	875 CYEVQIKAETRRILNNVGDWVKGPGTLLALMGASGAGKTTLLDCLAERVTMGVITGDLVNV---
...	
PDR5 N	228 GDDIKKHFPRGEVVVNAEADVHLPHLTIVETLVTVARLKTPONRIGVDRGKSYANHLLAEV-AMATY
PDR5 C	935 GIPRDKSFPRSIGYCCQQDHLKLTATVRESLRPSAYLRQPAE-VSIEEZKRYVEEVKILEMEKY
S B	
PDR5 N	292 GLSHTRNTKVGVNDIVRGVSGGERKRSVIAEVSVICGSKFPQCW-DAATRGLDSATALEFIRALNQ
PDR5 C	999 A-----DAVVGVAGECLNVEQRKRITIGVELTAKPKLLVFLDEPTSGLDSQTAWNSICQLMXXXXLA
...	
PDR5 N	356 DISNTSATVAIYQCSQDAYDLPNPKVCVLDDGYQIYYPADAKKYYFEDHGYC
PDR5 C	1058 NHQGAILC-TIHQPSA1MQLQEPDRL-----LPWQRGGKTV-YPGDLGEGC
...	

accompanied by an overexpression of the encoded polypeptides. Different subcellular fractions from *pdr1* mutants, enriched either in soluble proteins, in mitochondrial and ribosomal components, or in plasma membranes, were analyzed. Fig. 7A shows that in the plasma membrane-enriched fractions from the *pdr1-6* mutant, a strong fuzzy band of about 160 kDa is overexpressed. The soluble and mitochondrial/ribosomal fractions from the *pdr1-6* mutant did not show any difference when compared with the parental strain pattern. Overexpression of the 160-kDa plasma membrane protein(s) is also observed in the *pdr1-3* mutant (Fig. 7B). The replacement of a mutant (= resistance) *pdr1* allele by a null (= hypersensitivity) *pdr1*Δ allele results in the disappearance of the fuzzy band (Fig. 7B). Microsequencing of the NH₂ terminus of the overexpressed band from a *pdr1-6* mutant yielded two distinct sequences with similar intensity. The first one, P-E-A-K-L-N-N-N-V-N-D, corresponds to the amino acid sequence deduced from the 5'-terminal sequence of the *PDR5* gene without the N-terminal methionine. The second sequence detected, D-D-V-P-A-I-E-V-V-G-N-K-F-F-Y, is identical, over the first 15 amino acid residues, to the

sequence predicted for the glycoprophospholipid-anchored surface glycoprotein GAS1 (Nuoffer *et al.*, 1991), also known as GGP1 (Vai *et al.*, 1991), in its mature form depleted of the signal peptide.

DISCUSSION

The yeast and mammalian genes *PDR5* and *MDR1* share the capacity of conferring multidrug resistance upon amplification (Leppert *et al.*, 1990). In this study, we demonstrate that the corresponding proteins share structural homology and belong to the ABC superfamily (review by Higgins (1992)). In particular, PDR5 encodes a putative ATP-dependent plasma membrane protein resembling the mammalian transporter for drug efflux, termed the P-glycoprotein, encoded by *MDR1*. The ABC transporter that most resembles PDR5 is the recently described yeast *SNQ2* gene product, which also confers multidrug resistance upon amplification (Servos *et al.*, 1993). The disposition of the two hydrophobic regions, with six predicted transmembrane spans each, and of the two hydrophilic nucleotide binding folds of PDR5 mirrors that of the major eukaryotic four-domain ABC proteins.

		Walker A	ABC-Signature	Walker B
YEAST drug resistance	SNQ2 (N)	186-GEMILVLGRPGAGCASSYLEVY	109-VSGGERENVSIAARALAAKGSIT-Y-CWDEHATRGGLD	
YEAST pleiotropic drug resistance PDR5 (N)		186-GEMILVLGRPGAGCASSYLEVY	109-VSGGERENVSIAJVSICGSKPQ-CWDEHATRGGLD	
YEAST pleiotropic drug resistance PDR5 (C)		898-GTTLTALMGAGGAGCTTLLDCI	1010-LWVQGRERLTIGVELTARPFLVFLDEPTSGLD	
YEAST drug resistance	SNQ2 (C)	882-GTNTALMGAGGAGCTTLLNLY	993-LWVQGRERLISIGVELVAKPDILLFLDEPTSGLD	
YEAST permease ?	ADP1	416-GQILALMGRGGAGCTTLLDIL	330-IBGGERENVSIACLVTSPLV-L-PDEPTSGLD	
YEAST sensitivity	STE6 (N)	388-GQPTPIVGRGGAGCTTLLMIL	306-LSGGGQQQAVIARAFIRDTPIL-PDEATVSLD	
	STE6 (C)	1080-GQTLXIGEBSGAGCTTLLVLL	1190-LSGGGQGRCLIAARALLSRSKIL-ILDECTVSLD	
BACTERIA periplasmic permeases	CONSENSUS	49-GEVILALVPGPSSGAGCTTLLRII	183-LSGGGQQQAVIARALAJIPTVIL-LLDDEPTSGLD	
DROSOPHILA eye pigment	WHITE	192-GHLLAVRGGGGAGCTTLLMIL	230-LSGGERERLIAJASALTOPLL-ICDEPTSGLD	
DROSOPHILA eye pigment	BROWN	89-GDLIAILGGGGAGCTTLLMIL	167-LSGGERERLISLAEHLITDPIFL-PCDEPTSGLD	
PLANT chloroplast	mbpX	28-PGLIALLGPGGGAGCTTLLRIV	133-LSGGGQGRVALARSLAIQPDFL-LLDDEPTGALD	
RAT histocompatibility complex	mtp1	308-GKVTALVPGPSSGAGCTTVAALL	620-LSGGGQGRQAVALARALIREPFL-ILDDATVSLD	
HUMAN cystic fibrosis	CFTR (N)	481-GQLLAVAGGAGCTTLLMIL	848-LSGGGQGRISLARAVTEDADLY-LLDDEPTGALD	
HUMAN cystic fibrosis	CFTR (C)	1237-GQEVLLGAGGAGCTTLLSAY	1346-LSGGGQQLMCLARSLAKIL-LLDDEPTGALD	
HUMAN multidrug resistance	MDR1 (N)	420-GQTVALVPGPSSGAGCTTQVLM	931-LSGGGQGRQIAARALVREPKIL-LLDDEATVSLD	
HUMAN multidrug resistance	MDR1 (C)	1063-GQTLALVPGPSSGAGCTTQVLL	1176-LSGGGQGRQIAARALVREPKIL-LLDDEATVSLD	..

FIG. 4. Alignment of the A and B regions from the ATP-binding domains of PDR5 and of some members of the ABC transporters superfamily. The NH₂-terminal (N) and COOH-terminal (C) A and B regions of PDR5 are aligned with the corresponding regions of the yeast SNQ2 (Servos *et al.*, 1993), ADP1 (Purnelle *et al.*, 1991), and STE6 (McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989) transporters, with a consensus for bacterial periplasmic permeases (Mimura *et al.*, 1991) with the *Drosophila* eye pigments White (O'Hare *et al.*, 1984) and Brown (Dreesen *et al.*, 1988), with the plant chloroplast mbpX protein (Umesono *et al.*, 1988), with the rat major histocompatibility complex transporter mtp1 (Deverson *et al.*, 1990), and the human cystic fibrosis CFTR (Riordan *et al.*, 1989) and multidrug resistance MDR1 protein (Raymond and Gros, 1989). The amino acid residue number in each protein is indicated at the beginning of each sequence. The alignment was done by the program CLUSTAL (Higgins and Sharp, 1988). Asterisks indicate identical residues; points indicate conservatively substituted amino acids.

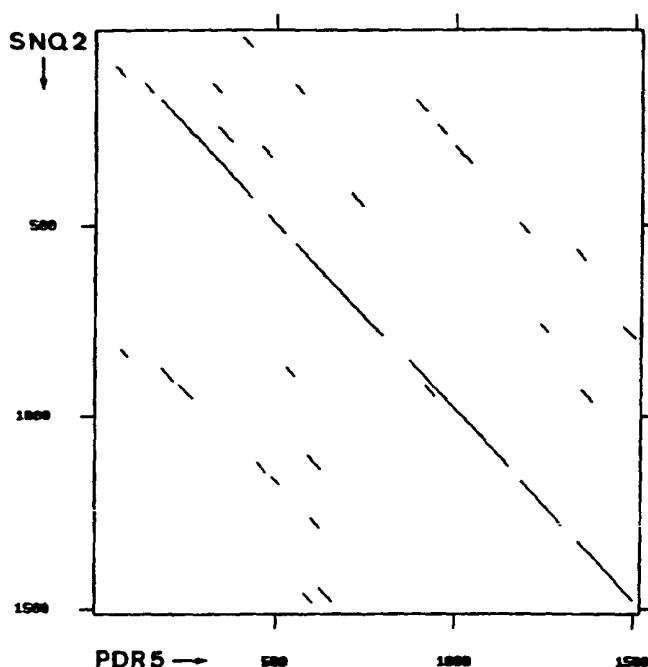


FIG. 5. Graphic comparison of the yeast PDR5 and SNQ2 proteins. The comparison plot was generated by the MICROGENIE program of Beckman, with a window of 30 residues with a minimal percentage of matches of 30. Numbering on the abscissa and ordinate axis refers to amino acids residues of the PDR5 and SNQ2 proteins, respectively.

PDR5 starts with an NH₂-terminal hydrophilic region followed by the first hydrophobic tract, whereas in P-glycoprotein (P-GTR), the hydrophobic domains precede the

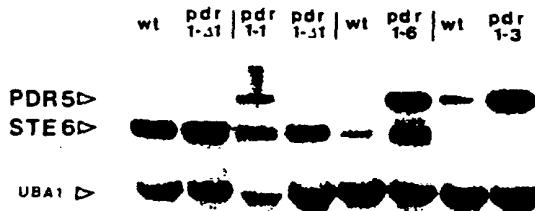
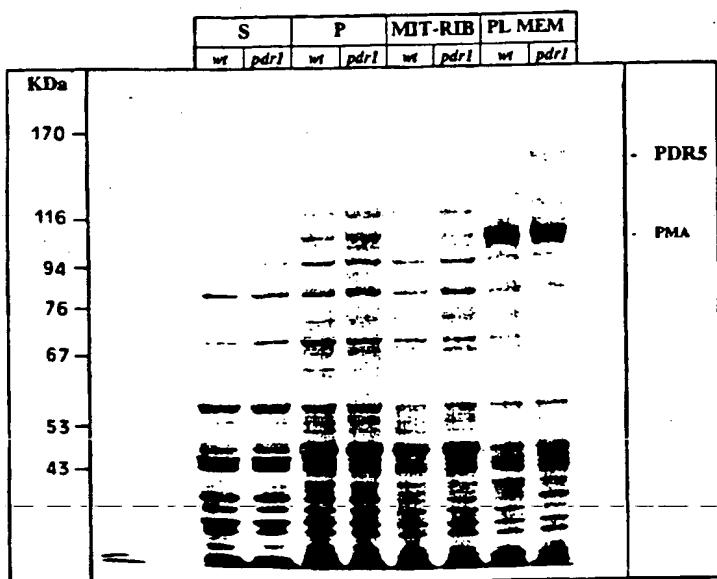


FIG. 6. Northern blot analysis of PDR5 and STE6 transcripts in pdr1 mutants. Thirty μ g of total RNA were run in each well of a 1% agarose-formaldehyde gel, transferred to nitrocellulose membranes, and hybridized to probes specific for PDR5 (4.7-kb *Pvu*II fragment from plasmid pDR3.3) and for STE6 and UBA1 (3.8-kb *Bam*HI fragment from plasmid pUC-STE6). Lanes 2 and 1, *pdr1*-6 mutant BOR2-XI and parental strain D286-2A; lanes 4 and 3, *pdr1*-3 mutant DRI9-T8 and parental strain IL125-2B; lane 6, D1 (*pdr1* Δ :*URA3*) strain obtained by disruption of *PDR1* in the wild type host 22295-C (lane 5); lane 8, D1-1 (*pdr1* Δ :*URA3*) strain obtained by disruption of *PDR1* in the *pdr1* Δ host US53-31D (lane 7). The UBA1 gene transcript, co-hybridized by the STE6 probe, is considered as control for general mRNA levels.

nucleotide binding folds. On the other hand, the "inverted" order of PDR5 is also observed in three ABC transporters, composed of only one hydrophobic and one hydrophilic domain, the *Drosophila* White and Brown eye pigment transporters and the yeast ADP1 permease, and, in the recently described four-domain yeast, SNQ2 protein, toward which the best similarity scores for PDR5 are obtained. It has been previously noted that the inverted domain order could eventually be related to import functions, as opposed to export functions (Juranka *et al.*, 1989). Although transport studies must still be undertaken for the PDR5 protein, we favor the hypothesis of PDR5 being involved in drug efflux, *in vivo* of

A



B

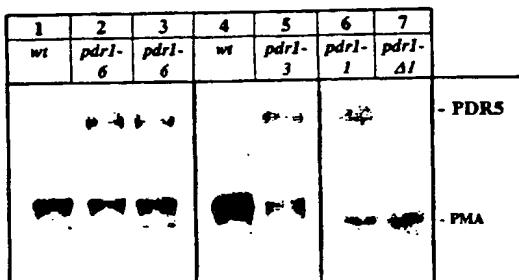


FIG. 7. Overexpression of 160-kDa membrane protein(s) in *pdr1* mutants. Seventy-five μ g of protein fractions prepared as described under "Experimental Procedures" were run on a 6-9% acrylamide gradient gel (3.5 h at 45 mA) and stained with Coomassie Blue. A, comparison of different protein fractions from the *pdr1-6* mutant BOR2-XI (*pdr1*) and its parental strain D286-2A (wt). Samples analyzed are: soluble protein fractions (S) and crude membrane pellets (P) obtained after high speed centrifugation, mitochondria/ribosome-enriched (MIT-RIB) and plasma membrane-enriched (PL MEM) fractions obtained after selective acid precipitation. B, details of high molecular weight plasma membrane proteins from the strains: parental D286-2A (wt; lane 1) and derived *pdr1-6* mutant BOR2-XI (lane 2), non-isogenic *pdr1-6* mutant US89-13B (lane 3), parental IL125-2B (wt; lane 4) and derived *pdr1-3* mutant DRI9-T8 (lane 5), *pdr1-1* mutant US53-31D (lane 6) and derived *pdr1Δ:UR43* disrupted strain D1-1 (lane 7). The overexpressed bands containing PDR5 and, as reference, the plasma membrane ATPase (PMA) are indicated.

the observation that PDR5 overexpression provokes drug resistance (Leppert *et al.*, 1990).

The two ATP-binding domains of PDR5 do not perfectly fit to all standard attributes of ABC cassettes (reviews by Ferro Luzzi Ames and Lecar (1992) and Thomas and Pedersen (1993)). Yet, the following peculiarities of PDR5 are also observed in the yeast SNQ2 protein. The most striking divergence is the replacement by a cysteine of the strictly conserved lysine G₁XGKT of the first nucleotide binding fold. Cysteine residues, possibly of the Walker A region from the P-glycoprotein, have been postulated to play a role in ATP hydrolysis, since sulphydryl-reactive reagents inhibit ATPase activity (Doige *et al.*, 1992). On the other hand, the conserved lysine has been proposed either to contribute to the conformation

of the phosphate binding loop, to interact directly with phosphate groups of the bound nucleotide, or even to intervene in catalysis (Saraste *et al.*, 1990; Tian *et al.*, 1990; Reinstein *et al.*, 1990). Its chemical modifications or substitutions modify ATPase activity of different nucleotide binding proteins (Sung *et al.*, 1988; Reinstein *et al.*, 1990; Tian *et al.*, 1990) but do not necessarily prevent ATP binding. Replacement of the lysine by arginine or alanine in either one of the nucleotide binding folds of the STE6 transporter impairs transport activity (Berkower and Michaelis, 1991). Similar replacements, by arginine or methionine, of the corresponding lysines of the mammalian P-glycoprotein decrease drug transport but do not affect the capacity to bind ATP (Azzaria *et al.*, 1989). If PDR5 catalyzes ATP hydrolysis-dependent drug efflux like its mammalian counterpart and if the conserved lysine is a strict requirement for ATPase activity, PDR5 would be able to support transport activity with only one functional ATP hydrolysis domain. In contrast, the two sites for ATP binding and hydrolysis are required for transport function of the P-glycoprotein (Azzaria *et al.*, 1989). It has been recently shown that the P-glycoprotein is bifunctional and exhibits both ATP hydrolysis-dependent drug transport and ATP binding-dependent ion channel activities. Moreover the mutation of the "Walker's lysine" to arginine in either one of the nucleotide binding folds abolished the function of drug transport but not the channel activity (Gill *et al.*, 1992). A non-hydrolytic, perhaps allosteric, mechanism of ATP dependence has also been recently proposed for activation of the cystic fibrosis chloride channel, CFTR (Quinton and Reddy, 1992). The mutation of the Walker's lysine, to methionine, in the second nucleotide binding fold of CFTR does not abolish Cl^- conductance, while the corresponding mutation in the first domain prevents maturation of CFTR and accordingly channel activity (Cheng *et al.*, 1990; Gregory *et al.*, 1991; Anderson *et al.*, 1991). These results suggest that the two nucleotide-binding domains of CFTR may be functionally not equivalent. Therefore, the concept that structurally homologous ABC proteins or domains might exert different, active or passive, transport functions by using ATP differently, via allosteric binding or hydrolysis, has been put forward. Investigations of the transport activities of the PDR5 polypeptide, which displays a natural and rare variant of a nucleotide binding fold, might help to resolve the relative importance of the two ATP-binding domains, with respect to both active transport and channel activities.

Another particular feature of the PDR5 ATP-binding cassettes, also observed in SNQ2, allows an interesting comparison with the cystic fibrosis factor CFTR. The COOH-terminal ABC cassette of PDR5 contains a "linker peptide" sequence (LNVEQ...) rather divergent from the common consensus LSGQQ... (Shyamala *et al.*, 1991; Ferro-Luzzi Ames and Lecar, 1992). The nonconserved asparagine in position 2 of this PDR5 linker peptide corresponds exactly to a CFTR mutation (S549N) associated with cystic fibrosis in man (Cutting *et al.*, 1990). Furthermore, the second most common cystic fibrosis mutation is the replacement of a conserved glycine by an aspartate in position 4 of the linker peptide from the first nucleotide binding fold of CFTR (G551D; Cutting *et al.*, 1990). In the corresponding position, the COOH-terminal ABC cassette of PDR5 displays a glutamate, thus a negatively charged residue like the cystic fibrosis-associated amino acid.

We have detected the PDR5 protein in a plasma membrane-enriched fraction. In various wild type strains, PDR5 is a minor membrane component, undetectable by Coomassie Blue staining of plasma membrane fractions. A massive over-

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production of the PDR5 mRNA and protein takes place in some mutants of the transcription regulator PDR1 selected by their multidrug resistance. The overexpressed PDR5 protein appears as a broad and fuzzy or multiple band of an average size of 160 kDa, reminiscent of the polydispersity observed for the cystic fibrosis protein CFTR (Sarkadi *et al.*, 1992) and for the P-glycoprotein (Greenberger *et al.*, 1987), and possibly attributed to differential glycosylation and/or other post-translational modifications. In addition, proteins other than PDR5 could also be present in the 160-kDa fuzzy, multiple band. The STE6 protein could be expected to be overexpressed in the 160-kDa band of the *pdr1-6* mutant, which overexpresses the STE6 mRNA. The lack of detection of the STE6 protein in the 160-kDa band sequenced could be due to modifications of the NH₂ terminus blocking the protein toward sequencing. On the other hand, the glycoprotein GAS1/GGP1 was detected in the overexpressed 160-kDa band; whether this major peripheral protein undergoes regulation by PDR1 remains to be further explored.

The yeast *pdr1* mutants represent, to our knowledge, the first case of established regulatory mutations provoking amplification of multidrug resistance-like transporters. The remarkable overexpression of the PDR5 protein in the *pdr1-3* and *pdr1-6* mutants provides a unique tool for isolation and further characterization of this protein. It also provides some hints for studying the mechanism of transcriptional regulation of PDR5 by the PDR1 protein. These experimental features are unique to yeast and may serve as a model to study the increased transcription of P-glycoprotein genes, which is nearly always observed in mammalian multidrug-resistant cell lines (reviews by Schinkel and Borst (1991), Bradley *et al.* (1988), and Juranka *et al.* (1989)). Unknown regulatory mechanisms controlling the expression of the P-glycoprotein must thus play a primary role in the establishment of mammalian drug resistance. The interest of exploring the regulation of MDR-like genes encompasses the problem of multidrug resistance since, even in normal mammalian tissues, the expression of the P-glycoprotein, as well as of the cystic fibrosis protein CFTR, seems to be highly and coordinately regulated (Trezise *et al.*, 1992). This common regulation is reminiscent of the transcription regulation by PDR1 of both the yeast PDR5 and STE6 genes.

The MDR1 gene promoter has been reported to include heat shock consensus elements and to be induced in response to temperature elevations and to other chemical stress-inducing agents (Chin *et al.*, 1990; Kioka *et al.*, 1992; Miyazaki *et al.*, 1992). Interestingly, the promoter of the PDR5 gene contains a tandem repeat of heat shock consensus elements, which might suggest some analogies between the yeast PDR5 and mammalian MDR genes at the level of promoter regulation.

In conclusion, the uncovering in yeast of regulatory elements, like PDR1, controlling the expression of P-glycoprotein-like genes, such as PDR5 and STE6, might provide a model for the search of related circuits that would control, in mammals, the expression of genes encoding ABC transporters like the multidrug-resistant protein and the cystic fibrosis factor.

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